Short Communication

Desmutagenic Activity of Peroxidase on Autoxidized Linolenic Acid

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Received November 26, 1979

In a preceding paper,¹⁾ we reported that autoxidized fatty acids showed mutagenicity when they were assayed by the Ames test in a restricted range of the concentration after sonication with certain detergents or proteins. With a view to secure food containing autoxidized fats and oils, studies on screening of desmutagens on autoxidized fatty acids have been made as to various vegetable and fruit samples. The word "desmutagen" was first used by Morita *et al.*^{2,3)} to demonstrate destructive activity of vegetable factors on the mutagenicity of pyrolysis products of tryptophan.

We report here that juices, prepared from some vegetables belonging to the family *Cruciferae* were found to possess desmutagenic activities on autoxidized linolenic acid (AOL). This factor was proved to be a peroxidase contained in these juices.

Linolenic acid (free or methyl ester), autoxidized at 40°C for 40 hr, and the AOL obtained about 2,000 to 2,500 P.O.V. by Wheeler method⁴⁾ was sonicated with Tween 20 by the method described in the previous paper.¹⁾

Vegetable and fruit samples were homogenized and centrifuged at $9,000 \times g$ for 30 min. The supernatant fractions (juice samples) separated were stocked at -40°C.

The assay method of desmutagenic activity of the juice on AOL was as follows;

The pretreated AOL resuspended in Tween 20 was added to 0.5 ml of juice sample contain-

ing 200 µg/ml of ampicillin for sterilization and the mixture was incubated at 30°C for 30 min. The mutagenicity of the above mixture was assayed by the method of Ames *et al.*⁵⁾ using *Salmonella* TA 98 as a tester strain. The desmutagenicity was estimated from the number of His⁺ revertant colonies reduced by the presence of each juice sample.

Table I shows desmutagenic effectiveness of

TABLE I. DESMUTAGENIC ACTIVITIES OF VARIOUS
VEGETABLE AND FRUIT JUICES

Sample ^a	Mean number of His ⁺ revertants per plate	Desmutagenic capacity
Broccoli	86	++
Cabbage	98	++
Peamann	57	++
Lettuce	235	-
Celery	270	-
Potato	185	±
Sweet potato	210	-
Taro	176	±
Radish	95	++
Pasley	273	-
Onion	255	-
Welsh onion	173	±
Red cabbage	86	++
Chinese cabbage	73	++
Honewort	265	-
Pumpkin	212	-
Cucumber	268	-
Tomato	110	+
Chayote	55	++
Egg plant	215	-
Shungiku	195	±
Cauliflower	98	++
Burdock	110	+
Carrot	195	-
Leaf-beet	107	+
Spinach	278	-
Small turnip	73	++
Leaf musturd	67	++
Ginger	95	++
Indian lotus	255	-
Kidney bean ^b	115	+
Mung bean sprouts	267	-
Shimeji mushroom	263	-
Grape	275	-
Mandarin orange	315	-

Apple	255	—
Sand pear	272	—
Banana	132	+
Control (only AOL)	265	
None ^c	45	

^a Each juice sample contains 200 µg/ml ampicillin for sterilization of bacteria in the sample. This concentration of ampicillin was tested to have no effect on the mutagenic assay, since the tester strain TA 98 is ampicillin resistant.

^b Unripe sample.

^c Spontaneous revertants without AOL.

Other experimental conditions were described in the text.

various juice samples on the mutagenicity of AOL.

Results shown in Table I indicate that a number of samples have no effect or very moderate activity on reduction in the number of His⁺ revertant colonies, but some showed considerable effectiveness. Especially, broccoli, cabbage, peamann, radish, red cabbage, chinese cabbage, chayote, cauliflower, small turnip, leaf musturd and ginger showed remarkable effects.

Active factors detected in most of these juices were found to be enzymatic proteins that are

TABLE II. PEROXIDASE AND CATALASE ACTIVITY OF VARIOUS VEGETABLE AND FRUIT JUICES

Sample	Peroxidase ^a (u/ml)	Catalase ^b (u/ml)
Broccoli	104	26
Cabbage	69	28
Peamann	0	35
Lettuce	0	120
Celery	3	182
Potato	0	3
Sweet potato	0	6
Taro	0	75
Radish	20	48
Pasley	0	115
Onion	0	12
Welsh onion	36	38
Red cabbage	100	52
Chinese cabbage	12	35
Honewort	0	90

Pumpkin	0	58
Cucumber	0	140
Tomato	85	6
Chayote	25	145
Egg plant	0	0

Shungiku	0	12
Cauliflower	30	72
Burdock	22	0
Carrot	0	0
Leaf-beet	9	0

Spinach	0	125
Small turnip	130	115
Leaf musturd	9	70
Ginger	64	145
Indian lotus	0	0

Kidney bean ^c	29	0
Mung bean sprouts	5	0
Shimeji mushroom	0	0
Grape	0	0
Mandarin orange	0	12

Apple	0	12
Sand pear	0	0
Banana	9	115

^a The peroxidase activity was determined by the modified method of Trinder.⁶⁾ The enzyme reaction was coupled with 4-aminoantipyrine and *N,N*-dimethylaniline.

^b The catalase activity was determined by the modified method of Kageyama,⁷⁾ in which the enzyme reaction was coupled with acetylacetone, methanol and ammonia.

^c Unripe sample.

heat-labile, non dialyzable and sensitive to protease.

It was also found that many desmutagenic vegetable samples belong to family *Cruciferae*. Since these vegetables are known to possess more or less peroxidase and catalase, we examined these enzymes as for their possibility of involvement in the desmutagenic effect on AOL. The peroxidase activity was determined by the modified method of Trinder⁶⁾ and the catalase activity was measured by the modified method of Kageyama.⁷⁾

Table II shows assay results on peroxidase and catalase activities in various juice samples.

As shown in Tables I and II, most of the samples having desmutagenic activities showed strong activity of peroxidase, suggesting that the desmutagenicity has some connection with

this enzyme. On the other hand, catalase seemed to be not so effective as to the desmutagenicity. The desmutagenicity of these enzymes was also studied with pure samples of peroxidase of horseradish as well as catalase of bovine liver (Table III).

TABLE III. EFFECT OF PEROXIDASE AND CATALASE ON THE MUTAGENICITY OF AOL

Enzyme	Guaiacol μg/plate	Mean number of His ⁺ revertants per plate
Peroxidase 10 u/plate	0	262
	50	98
	100	63
	300	61
Catalase 500 u/plate	0	275
	50	250
	100	225
	300	225
None	0	253
	50	250
	100	215
	300	217

Both peroxidase and catalase used were commercial preparations obtained from Sigma Chem. Co., Ltd. Their potency were respectively 75 unit per mg solid (horseradish type I) and 2,000 unit per mg solid (bovine liver). Each enzyme and guaiacol were added to the AOL suspension, incubated for 30 min at 30°C, then the mutagenicity was assayed by the standard Ames method.⁵⁾

In Table III, it is shown that when AOL was incubated solely with each enzyme, no effect was observed as to the mutagenicity of AOL. However, if guaiacol, a hydrogen donor, was added in combination with peroxidase, a net desmutagenicity on AOL was marked. In the course of incubation period of AOL with

peroxidase plus guaiacol, brown color appeared as the incubation proceeds, but no color appeared if guaiacol was not present. In the case of catalase, neither desmutagenicity nor any color reaction was observed whether guaiacol was added or not.

Horseradish peroxidase has reported to possess high substrate specificity. Among various peroxides, only dihydrogen, ethyl hydrogen and methyl hydrogen have been found to act as primary substrates.⁸⁾

We like to point out that AOL is a good substrate for horseradish peroxidase.

The fact that the juice samples having peroxidase activity don't require guaiacol for the desmutagenicity seems to suggest that they themselves may contain certain hydrogen donor(s).

The desmutagenicity of peroxidase on AOL was also clearly confirmed using the rec⁻ assay system developed by Kada *et al.*⁹⁾

Acknowledgments. We are grateful to Dr. T. Kada of National Institute of Genetics for valuable discussion.

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